

APPLICATION

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TITLE: IMMUNOTHERAPY OF CANCER WITH ALLOGENEIC LYMPHOCYTES

APPLICANT: SHIMON SLAVIN

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Francisco Robles

Francisco Robles

FIELD OF THE INVENTION

This invention relates to methods of eradicating residual tumor cells following surgical intervention, chemotherapy and/or radiotherapy. The methods involve autologous bone marrow transplantation followed by administration of
5 allogeneic-peripheral blood leukocytes. More particularly, this invention relates to use of HLA-compatible allogeneic peripheral blood lymphocytes (PBL), following autologous bone marrow and/or peripheral blood stem cell transplantation, to induce a graft-versus-malignant cell response. Such a response is instrumental in prevention of disease relapse.

BACKGROUND OF THE INVENTION

10 Patients with malignant hematological disorders resistant to conventional doses of chemotherapy and/or radiation may be treated by autologous or allogeneic bone marrow transplantation. Bone marrow transplantation (BMT)
15 makes it possible to administer to patients with resistant disease high, "supra-lethal," combinations of chemotherapy and radiation, ignoring the irreversible toxicity of such therapeutic combinations on the normal bone marrow compartment. Nevertheless, such "debulking" of a patient's tumor(s) can leave a fraction of residual malignant cells that may lead to disease relapse. Several lines of
20 evidence suggest that a significant proportion of the beneficial effect of allogeneic BMT (i.e., BMT from an individual not genetically identical to the host patient) stems from cell-mediated interactions of immune cells of donor origin against

residual tumor cells in the host that have escaped the chemoradiotherapy debulking regimen. .

Following allogeneic BMT, the incidence of relapse is significantly lower in leukemia patients with clinical manifestations of acute or chronic graft versus host disease-(GVHD), as compared with patients with no GVHD, indicating that immune-mediated allogeneic interactions of immunocompetent cells of donor origin against the host are also accompanied by graft vs. leukemia (GVL) effects. Weiden et al., N. Engl. J. Med. 300: 1068 (1979); Weiden et al., N. Engl. J. Med. 304: 1529-33 (1981); Weiden et al., Transplantation 13: 248-51 (1981); Barrett et al., Blood 74: 862 (1989); Sullivan et al., Blood 73: 1720 (1989); Horowitz et al., Blood 75: 555 (1990); Slavin et al., Bone Marrow Transplant. 6: 155-61 (1990).

Higher relapse rates seem to occur in patients undergoing allogeneic BMT with T-lymphocyte depletion for prevention of GVHD, compared to recipients of non-T cell depleted marrow allografts, regardless of the severity of GVHD. Horowitz et al., Blood 75: 555 (1990); Slavin et al., Bone Marrow Transplant. 6: 155-61 (1990); Goldman et al., Ann. Inter. Med. 108: 806-14 (1988); Ringden and Horowitz, Transplant. Proc. 21: 2989-92 (1989); Goldman et al., Ann. Int. Med. 108: 806 (1988). Likewise, relapse rates in patients with acute leukemia or chronic myeloid leukemia reconstituted by bone marrow grafts obtained from an identical twin (syngeneic grafts) are significantly higher than in those reconstituted by bone marrow cells obtained from an HLA-identical but non-syngeneic sibling. Ringden and Horowitz, Transplant. Proc. 21: 2989-92 (1989). Similarly, relapse rates following

transplantation of the patient's own (autologous) marrow, even following adequate purging in vitro for elimination of residual leukemia cells, are significantly higher than following allogeneic BMT. Armitage, Curr. Opinion in Hematol. 1993: 240-45 (1993). Thus, the less-than optimal results with autologous BMT (ABMT) are

5 similar to the results seen with syngeneic marrow transplantation. All of the above data suggests that in practical terms GVHD or GVHD-potential correlates with a lower incidence of relapse.

Allogeneic donor cells may also play a role against lymphoma, as shown in experimental animals, Slavin et al., Cancer Immunol. Immunother. 11: 155-58 (1981), and humans. Phillips et al., J. Clin. Oncol. 4: 480-88 (1986); Ernst et al., 10 Proc. of the 4th International Conference on Lymphoma, Lugano 1990, Abstract #P35; Chopra et al., J. Clin. Oncol. 10: 1690-95 (1992). As shown in experimental animals, graft-versus-tumor effects (GVT), similar to graft-versus-leukemia effects (GVL), may occur following BMT, independently of GVHD. Moscovitch and Slavin, 15 J. Immunol. 132: 997-1000 (1984).

Although GVHD-associated anti-leukemia effector mechanisms may be of benefit in BMT, nevertheless GVHD represents one of the major obstacles in allogeneic BMT, even among perfectly HLA-matched siblings. Acute GVHD develops in the majority of recipients of allogeneic BMT, with clinically significant 20 manifestations in 26-64% of the recipients despite optimal post-transplant immunosuppressive prophylaxis. Storb et al., Blood 73: 1729 (1989). Chronic GVHD may occur in up to 45% of long term survivors. Storb et al., Blood 73: 1729 (1989).

There is no satisfactory therapy for patients with established GVHD and hence patients with severe manifestations of acute or chronic forms of the disease are prone to develop multisystem complications that may require frequent hospitalizations, leading to poor quality of life and occasionally serious or even fatal complications.

GVHD following allogeneic BMT can be prevented by adequate pretransplant T-lymphocyte depletion, using no post-transplant anti-GVHD prophylaxis. Reisner et al., In: Slavin, S (ed.), Tolerance in Bone Marrow and Organ Transplantation. Elsevier, Amsterdam (1984), p. 293; Waldmann et al., Lancet 2: 483-85 (1984); Slavin et al., Transplant. Proc. 17: 465-67 (1985). BMT without GVHD represents a better tolerated procedure that may necessitate shorter hospitalization with superior subjective immediate outcome following allogeneic BMT. In addition, the quality of life of long-term survivors without GVHD is clearly better than for those patients with severe acute or chronic GVHD.

Unfortunately, the advantages of GVHD-free-allogeneic BMT are counterbalanced by other serious complications due to untoward effects of T-lymphocyte depletion, such as increased incidence of graft rejection, occurring in 10-30% of recipients, as well as increased rates of tumor relapse. Martin et al., Bone Marrow Transplant. 3: 445 (1988); Kernan et al., Transplantation 43: 842 (1987); Waldmann et al., Lancet 2: 483-85 (1984); Slavin et al., Transplant. Proc. 17: 465-67 (1985). Consequently, there seems to be no clear evidence to date for a significant overall benefit of GVHD prevention by T-lymphocyte depletion.

Clearly, it would be a significant advance in the art to be able to combine the benefits of minimal or controllable GVHD risk following ABMT or ASCT with induction of graft-versus-malignant cell response that may be associated with GVHD following allogeneic BMT.

5

SUMMARY OF THE INVENTION

10 The present invention includes a method of treating a human cancer patient who has undergone a malignant cell debulking regimen. Generally the patient is considered to be at risk for disease relapse due to a population of residual malignant cells that may remain viable in the patient following the debulking procedure. A sample of stem cells, taken from the patient, is obtained and determined to be suitable for autologous transplantation into the patient. This sample is used to perform an autologous transplant of the patient, i.e, infusing the patient's stem cells back into the patient. The patient is then monitored until the patient is partially hematopoiesis recovered but is not fully immune-reconstituted. 15 Then, the patient is administered an HLA-compatible, allogeneic peripheral blood leukocyte preparation having lymphocytes, in a regimen that causes a clinically significant graft-versus-malignant cell response. The patient is then monitored for levels of malignant cells deriving from any residual malignant cells that might have been present following the original debulking procedure. This monitoring 20 may constitute one or more molecular or cellular assays to detect or quantify

malignant cells, may constitute a monitoring program to detect clinical signs of relapse, or any combination of these monitoring methods.

As used herein, a clinically significant response permits, for example, the patient to avoid relapse, substantially prolongs the time to relapse or otherwise engenders a beneficial condition that significantly prolongs life. A graft-versus-malignant cell response is not clinically significant unless there is some clinical benefit to the patient. Evidence for a clinically significant response may include, for example, absence or delay of relapse, evidence for elimination of minimal residual disease, i.e., elimination of disease-specific markers or, where appropriate, elimination of markers directed to host-specific cells.

The regimen for administration of an HLA-compatible leukocyte preparation including lymphocytes may comprise the following steps in sequence:

- a) treating the patient by administration (e.g., infusion) of about 10^7 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic peripheral blood lymphocytes;
- b) monitoring the patient for indications of a graft-versus-malignant cell response; and
- c) if no or insufficient graft-versus-malignant cell response develops in the patient, escalating the treatment by performing at least one of the following procedures: (1) administration of a number of HLA-compatible, allogeneic peripheral blood lymphocytes greater than the number of lymphocytes administered in step (a); (2) administration of a number of HLA-compatible, allogeneic peripheral

blood lymphocytes at least as great as the number of lymphocytes administered in step (a), accompanied by administration of at least one T-cell-activating cytokine to said patient; (3) administration of HLA-compatible, allogeneic cytokine-activated lymphocytes (CAL) to said patient; and (4) administration of HLA-compatible, allogeneic CAL, accompanied by administration in vivo of at least one T-cell-activating cytokine to said patient. More than one of these procedures can be performed if no or insufficient graft-versus-malignant cell response develops in the patient following the first or subsequent procedure.

10 In an alternative embodiment, step (a) above can be augmented by administering, concomitant with the allogeneic lymphocytes, at least one T-cell-activating cytokine to the patient. Since the T-cell-activating cytokine is administered directly to the patient, the infused allogeneic lymphocytes are exposed to the cytokine in vivo.

15 The present invention also includes an alternative method of treating a human cancer patient who has undergone a malignant cell debulking regimen. Generally the patient is considered to be at risk for disease relapse due to a population of residual malignant cells that may remain viable in the patient following the debulking procedure. A sample of stem cells, taken from the patient,
20 is obtained and determined to be suitable for autologous transplantation into the patient. This sample is used to perform an autologous transplant of the patient, i.e., infusing the patient's stem cells back into the patient. The patient is then monitored

until the patient is partially hematopoiesis recovered but is not fully immune-reconstituted. Then, the patient is administered an HLA-compatible, allogeneic peripheral blood leukocyte preparation having lymphocytes, in a regimen that causes a mild graft-versus-host response. The patient is then monitored, as above,
5 ~~for levels of malignant cells deriving from any residual malignant cells that might~~
have been present following the original debulking procedure.

As used herein, the term "graft-versus-host response" includes but is not limited to the classic clinical symptoms of graft-versus host disease (GVHD), known to those having ordinary skill in the art. The term "graft-versus-host
10 response" also includes molecular or cellular responses that correlate with the clinical symptoms of GVHD or with the impending onset of the clinical symptoms of GVHD.

For the above-described alternative method, the regimen for administration of an HLA-compatible leukocyte preparation including lymphocytes
15 may comprise the following steps in sequence:

- a) treating the patient by administration of about 10^7 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic peripheral blood lymphocytes;
- b) monitoring the patient for indications of a mild graft-versus-host response; and
- 20 c) if no or insufficient graft-versus-host response develops in the patient, escalating the treatment by performing at least one of the following procedures: (1) administration of a number of HLA-compatible, allogeneic peripheral blood

lymphocytes greater than the number of lymphocytes administered in step (a); (2) administration of a number of HLA-compatible, allogeneic peripheral blood lymphocytes at least as great as the number of lymphocytes administered in step (a), accompanied by administration of at least one T-cell-activating cytokine to said patient; (3) administration of HLA-compatible, allogeneic CAL to said patient; and (4) administration of HLA-compatible, allogeneic CAL, accompanied by administration in vivo of at least one T-cell-activating cytokine to said patient. More than one of these procedures can be performed if no or insufficient graft-versus-host response develops in the patient following the first or subsequent procedure.

In an alternative embodiment, step (a) above can be augmented by administering, concomitant with the allogeneic lymphocytes, at least one T-cell-activating cytokine to the patient. Since the T-cell-activating cytokine is administered directly to the patient, the infused allogeneic lymphocytes are exposed to the cytokine in vivo.

In an alternative embodiment, the regimen for administration of an HLA-compatible leukocyte preparation including lymphocytes may comprise the following steps in sequence:

- a) administering to the patient about 10^7 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic peripheral blood lymphocytes and at least one T-cell-activating cytokine to the patient;;

- b) monitoring the patient for signs of a mild graft-versus-host response;
- c) if no or insufficient graft-versus-host response develops in the patient, administering about 10^7 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic CAL and at least one T-cell-activating cytokine to the patient;
- 5 d) monitoring the patient for signs of a mild graft-versus-host response;

Alternatively, CAL may be given in the initial infusion. In this case the regimen for administration of an HLA-compatible leukocyte preparation including lymphocytes may comprise the following steps in sequence:

- 10 a) administering to the patient about 10^5 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic peripheral blood lymphocytes in which at least some of the HLA-compatible, allogeneic peripheral blood lymphocytes are CAL, and at least one T-cell-activating cytokine to the patient;;
- b) monitoring the patient for signs of a mild graft-versus-host response;
- 15 c) if no or insufficient graft-versus-host response develops in the patient, administering about 10^5 cells/kg to about 10^9 cells/kg of HLA-compatible, allogeneic CAL and at least one T-cell-activating cytokine to the patient;
- d) monitoring the patient for signs of a mild graft-versus-host response;

20 In any of the above described methods, the cytokine can be, without limitation, any one or more of the following: interleukin 2 (IL2), interleukin 4 (IL4), interleukin 5 (IL5), interleukin 6 (IL6), interleukin 7 (IL-7), interferon alpha (IFN α),

interferon gamma (IFN γ) and tumor necrosis factor (TNF α). Any of these cytokines can be a native factor obtained from natural sources, a factor produced by recombinant DNA methodology, a chemically synthesized polypeptide or other molecule, or any derivative having the functional activity of the native factor.

5 ~~The stem cells used in the above-described methods~~ may be obtained from bone marrow, from the peripheral circulation, or, where appropriate, from fetal sources such as fetal tissue, fetal circulation and umbilical cord blood. Cancer patients treatable with the methods of the present invention are any patients having a pathological condition caused by malignant cells, including without limitation
10 leukemia, lymphoma, and breast cancer. The HLA-compatible allogeneic cells employed in the present invention preferably are fully HLA-matched with the patient. Alternatively the allogeneic cells may be at least haploidentical with the patient. If the allogeneic cells are derived from a sibling of the patient, the cells preferably are fully HLA matched with the patient, although some mismatch may be
15 tolerated. For example, the allogeneic cells from a sibling to the patient may, in some cases, be single HLA locus-mismatched. If the allogeneic cells are derived from an unrelated individual, preferably the cells are fully HLA matched with the patient.

 The present invention further includes an article of manufacture
20 comprising packaging material and a biological cell container within the packaging material. The packaging material contains a label or package insert indicating that

the biological cell container, and/or any contents therein, are to be used in any of the above-described methods for treating a human cancer patient.

BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 depicts the results of adoptive transfer of spleen cells obtained from lethally irradiated F1 mice transplanted with 10^7 syngeneic bone marrow cells and 10^5 BCL1 cells in addition to $20-30 \times 10^6$ PBL from allogeneic mice with (A, n=40) or without (B, n=40) concomitant in vivo rhIL2 treatment (12×10^4 IU \times 2/day for 5 days IP). A similar group received syngeneic (F1) PBL given with (C, n=20) or
10 without (D, n=20) rhIL2. A control group had adoptive transfer of spleen cells obtained from 10 untreated F1 recipients (E, n=20).

Figure 2 depicts the results of adoptive transfer of spleen cells obtained from lethally irradiated F1 mice reconstituted with 10^7 syngeneic bone marrow cells mixed with 10^5 BCL1 cells. Cell-mediated immunotherapy consisted of intravenous
15 administration of increasing numbers of C57BL/6 spleen cells: 1×10^6 (A, n=10); 3×10^6 (B, n=10); 10×10^6 (C, n=10) and 30×10^6 (D, n=10). One of three experiments is shown.

Figure 3 depicts results of adoptive transfer of spleen cells obtained from BALB/c mice treated with cyclophosphamide (300 mg/kg IP), inoculated 24
20 hours later with 10^3 BCL1 cells, and one day later receiving 4×10^6 syngeneic, ASTA-Z-treated bone marrow cells. Immunotherapy consisted of a mixture of 20×10^6 allogeneic C57BL/6 spleen and lymph node cells either with (A, n=6) or without (B,

n=6) rhIL2 treatment in vivo (12×10^4 IU \times 3/day for 3 days IP). Recipients of a mixture of 20×10^6 syngeneic BALB/c spleen and lymph node cells treated with rhIL2 (C, n=7) and recipients of 10^3 BCL1 cells only (D, n=10) served as controls.

Figure 4 depicts the results of adoptive transfer of spleen cells obtained from lethally irradiated F1 mice inoculated with 10^5 BCL1 cells and 30×10^6 bone marrow cells pre-activated in vitro for 4 days with rhIL2; mice with no additional treatment (A, n=33), mice with in vivo rhIL2 treatment (12×10^4 IU \times 2/day for 5 days IP) (B, n=25); controls: recipients of 10^5 spleen cells obtained from untreated control mice inoculated with 10^5 BCL1 cells (C, n=30).

DETAILED DESCRIPTION OF THE INVENTION

The present inventor has employed allogeneic peripheral blood lymphocytes, alone or in combination with cytokine treatment in vivo or in vitro, for successful elimination of minimal residual disease following chemotherapy and/or radiotherapy. Appropriate treatment regimens have been circumscribed by studies in laboratory animals, and the treatment protocols have been further extended to human patients at high risk for disease relapse.

A spontaneous, transplantable murine B-cell leukemia/lymphoma of BALB origin (BCL1) was used to investigate elimination of minimal residual disease (MRD) following bone marrow transplantation. As used herein, MRD refers to a condition wherein residual malignant cells remain viable in a patient following a primary and/or metastatic tumor "debulking" regimen. The debulking regimen

may include surgical excision, chemotherapy or radiotherapy, or any combination of these approaches. Such treatment removes a significant fraction of malignant cells from the patient, but may leave a clinically significant number of residual malignant cells that put the patient at risk of relapse. The term "tumor" as used
5 herein includes all pathological conditions involving malignant cells; this can include "solid" tumors arising in solid tissues or organs as well as "liquid" tumors such as leukemias and lymphomas deriving from malignant transformation of hematopoietic cells.

10 In autologous bone marrow transplantation (ABMT) with human patients, an individual receives his or her own bone marrow cells by infusion following a tumor debulking procedure. Generally, the bone marrow cells are taken from the patient and preserved, for example by cryopreservation, prior to the debulking procedure. This permits an otherwise lethal debulking regimen to be employed, e.g., chemotherapy or radiotherapy that severely damages or destroys the
15 patient's bone marrow. Following the debulking procedure, the patient's bone marrow is reconstituted by stem cells present in the preserved sample of bone marrow.

Stem cells capable of reconstituting a patient's immune system can be obtained not only by direct extraction from the bone marrow, but also from the
20 patient's peripheral circulation following mobilization of such cells from the bone marrow. This can be accomplished by treatment of the patient with granulocyte colony stimulating factor (G-CSF) or other appropriate factors that induce

movement of stem cells from the bone marrow into the peripheral circulation. Following mobilization, the stem cells can be collected from peripheral blood by any appropriate cell apheresis technique, for example through use of a commercially available blood cell collection device as exemplified by the CS 3000® Plus blood cell collection device marketed by the Fenwal Division of Baxter Healthcare Corporation. Methods for performing apheresis with the CS 3000® Plus machine are described in Willams et al., Bone Marrow Transplantation 5: 129-33 (1990) and Hillyer et al., Transfusion 33: 316-21 (1993), both publications being incorporated herein by reference. Stem cells collected from the peripheral blood are termed herein "peripheral blood stem cells" (PBSC). The term "autologous stem cell transplantation" (ASCT) is used herein to refer to any infusion into a patient of that same patient's stem cells, derived from any appropriate source (e.g., bone marrow or peripheral circulation). As such, ABMT, where the autologous infused cells are extracted directly from the bone marrow of the patient, may be considered simply one form of ASCT.

It is possible to create an experimental regime in mice that simulates ASCT in humans. This is done through use of stem cell donors and recipients derived from a syngeneic strain of mice. In such strains, inbreeding has created a situation in which, for practical purposes, mice within the strain are genetically identical to each other. Such mice accept tissues and organs transplanted between individuals without evidence of immune rejection, in a manner analogous to acceptance of a patient's own cells following ASCT. Transplantation of bone

marrow-derived stem cells between such mice is referred to herein as "syngeneic bone marrow transplantation" (SBMT) and should be considered analogous to ABMT and ASCT in humans.

In the present experiments, mice received a lethal dose of total body irradiation (TBI) or, alternatively, a lethal dose of cyclophosphamide administered intraperitoneally. Bone marrow cells (BMC) were extracted directly from the bone marrow of syngeneic mice. In some cases, these BMC preparations were treated with mafosfamide (ASTA-Z) to simulate a "purging" procedure in which the patient's stem cells, prior to ASCT, are treated to remove or destroy at least a fraction of any contaminating malignant cells. One day after irradiation or treatment with cyclophosphamide, the mice received 10^7 syngeneic bone marrow cells by infusion into the lateral tail vein. To simulate MRD, 10^5 BCL1 tumor cells were added to the syngeneic BMC prior to SBMT.

Following SBMT, recipient mice received either allogeneic cell-mediated immunotherapy (Allo-CMI) or allogeneic cell-mediated cytokine-activated immunotherapy (Allo-CCI). Allo-CMI involved transfer of immunocompetent allogeneic lymphocytes (i.e., lymphocytes from a mouse strain other than that of the recipient mice) at various times and at various doses, administered post-SBMT. Generally these lymphocytes represented peripheral blood lymphocytes (PBL) or mixtures of donor spleen and lymph node cells. Allo-CCI involved transfer of allogeneic lymphocytes pre-activated in vitro with recombinant human interleukin 2 (rhIL2). As used herein, the term "CAL" refers to such "cytokine-activated

lymphocytes." In some experimental protocols, the Allo-CMI or Allo-CCI regimen was accompanied by simultaneous in vivo administration of rhIL2 to the recipient mice, in order to facilitate additional activation of the infused lymphocytes in vivo.

Failure to develop leukemia in primary recipients does not prove
5 elimination of all BCL1 cells, since active suppression of existing tumor cells can prevent development of overt disease in these animals. This has been documented following allogeneic BMT and rhIL2 therapy. Slavin et al., Cancer Immunol. Immunother. 11: 155 (1981); Slavin et al., Nat. Immun. Cell Growth Regul. 7: 180 (1988). To establish conclusive evidence for eradication of residual malignant cells,
10 spleen cells from the treated, or recipient, mice were adoptively transferred to secondary syngeneic recipients. If these secondary recipients failed to develop leukemia, it was judged that the original Allo-CMI- or Allo-CCI-treated mice were free of viable malignant cells, since as few as 1-10 cells have been shown to be capable of causing disease. Slavin et al., Cancer Res. 41: 4162 (1981); Cohen et al., J.
15 Immunol. 151: 4803-10 (1993).

The results of the SBMT experiments with mice suggested that effective immunotherapy of MRD can be achieved in vivo by cell therapy with alloreactive lymphocytes through an effect that can be further enhanced in vivo with a short course of intermediate-dose rhIL2. GVL-like effects were also induced
20 by infusion of CAL without causing any gross impairment of the hematopoietic capacity of BMC in lethally irradiated recipients. Moreover, GVL effects induced by allogeneic lymphocytes as well as CAL could be further enhanced by concomitant

rhIL2 therapy in vivo, most likely due to continuous in vivo activation of allogeneic effector cells against residual host malignant cells.

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The data further indicate that eradication of BCL1 can be accomplished before overt clinical manifestations of GVHD in the primary recipients would have occurred, since experiments showed that GVL-like effects against BCL1 cells were achieved within 1-2 weeks following administration of allogeneic lymphocytes. This implies that temporary engraftment of allogeneic effector cells may be sufficient to induce beneficial GVL effects against MRD, without the need for permanent residence of allogeneic effector cells, which may put the patient at risk for severe GVHD across major histocompatibility barriers. Moreover, as the time interval between ASCT and the Allo-CMI/CCI treatment increases, larger numbers of donor's PBL can be administered with less likelihood of severe GVHD. Slavin et al., J. Exp. Med. 147: 963 (1978); Slavin et al., Blood 80: 535a (1992).

As such, it is useful to administer an Allo-CMI or Allo-CCI regimen after the patient is partially hematopoiesis recovered following the original tumor debulking procedure/ASCT. This raises the likelihood that the allogeneic inoculum will be rejected by reconstituting host immune cells in due time. On the other hand, it is also desirable to undertake the Allo-CMI/Allo-CCI regimen prior to full immune reconstitution of the patient, since the likelihood of premature rejection of the allogeneic inoculum, prior to beneficial GVL/GVT effects, is thereby reduced. Since full immune reconstitution following ASCT in humans frequently requires up to one year, the patient can be monitored until a stable clinical condition (e.g.,

condition of stabilized blood counts) is attained, as indicated by acceptable levels of, for example, white blood cells (WBC), hemoglobin (Hb) and platelets. This condition of partial hematopoiesis recovery may be achieved in a matter of weeks following ASCT in humans, well before full immune reconstitution diminishes the
5 likelihood of successful Allo-CMI and/or Allo-CCI.

The Allo-CMI and Allo-CCI strategies developed in mice have been adapted to human patients in a variety of protocols undertaken by the present inventor in treating cancer patients at high risk of disease relapse. Cancer patients having acute myelogenous leukemia, chronic myelogenous leukemia, non-
10 Hodgkin's lymphoma and metastatic breast cancer have been treated with the methods of the present invention.

Two of the earlier-treated patients with acute myelogenous leukemia were given relatively low numbers (e.g., about 10^4 cells/kg) of allogeneic peripheral blood lymphocytes as a first dose, in an effort to minimize the risk of serious GVHD.
15 Thereafter, the patients were given escalating numbers of allogeneic peripheral blood lymphocytes at various time intervals to increase the chances of clinically significant graft-versus malignant cell response. Both of these early patients (patients 1 and 2 in Example 2, set out below) relapsed and died. Following this experience, it was apparent that administration of graded increments of PBL,
20 beginning with relatively low numbers, may not be effective. It was hypothesized that the initial low dose of allogeneic PBL produced only immunization without significant GVHD or graft-versus-malignant cell response. Thus, later, higher doses

of allogeneic PBL, which otherwise might be effective, are promptly rejected by the patient and rendered ineffectual for graft-versus-malignant cell activity. Therefore, in subsequent cases, more cells (e.g., at least about 10^7 cells/kg) were administered in the initial dose. With this initial higher dose of allogeneic cells following ASCT,

5 satisfactory results have been obtained in many patients within the treatment population to date.

Allogeneic cells preferably are chosen from human leukocyte antigen (HLA)-compatible donors. Generally, HLA-compatible lymphocytes may be taken from a fully HLA-matched relative such as a parent, brother or sister. However, as shown with patient No. 12 in Example 2, below, donor lymphocytes may be sufficiently HLA-compatible with the recipient to obtain the desired result even if a sibling donor is single-locus mismatched. If a donor is unrelated to the recipient, preferably the donor lymphocytes are fully HLA matched with the recipient.

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15 In a preferred embodiment of the present invention, an initial dose of at least about 10^7 HLA-compatible allogeneic lymphocytes/kg is given once the patient has achieved a clinically stable condition (e.g., stabilized blood counts). Preferably, the patient is also administered cytokine in vivo, concomitant with administration of the HLA-compatible lymphocytes. Preferably the cytokine is rhIL2 at a dose of about 6×10^6 IU of rhIL2/m²/day, by subcutaneous injection,
20 beginning on the same day as infusion of the allogeneic lymphocytes. Other appropriate cytokines may be used alone or in combination with rhIL2, as long as the desired enhancing effect is obtained. Such additional cytokines include, without

limitation, native or recombinant forms of IL6, IL7 and alpha and gamma-interferon (INF α , INF γ , respectively).

If GVHD fails to develop in a patient given these numbers of HLA-compatible allogeneic lymphocytes with concomitant in vivo cytokine, then the

5 treatment regimen is escalated. Preferably this is done by administering a second dose of allogeneic HLA-compatible lymphocytes preactivated in vitro with cytokine. Preferably the cytokine is rhIL2, although other appropriate cytokines may be used alone or in combination with rhIL2, as long as the desired activation in vitro is obtained. Such additional cytokines include, without limitation, native or
10 recombinant forms of IL6, IL7 and alpha-interferon (INF α), alone or in combination with IL2.

Prior to administration of the second dose of cells comprising CAL, and contingent on the particular status of an individual patient, cyclophosphamide (Cytosan) or other appropriate immunosuppressants may be administered to the
15 patient to avoid rejection of the second dose of cells. That is, the immunosuppressant is given in a dose effective for killing host T cells that might otherwise operate to reject the second allogeneic inoculum; the immunosuppressant may have the added benefit of eliminating potential host suppressor cell functions that can interfere with the GVT effects of the infused CAL.
20 Preferably, in vivo cytokine is administered to the patient concomitant with the second dose of cells (CAL).

Although the above-described preferred embodiment is presently recommended, it is to be understood that any combination of allogeneic peripheral blood lymphocytes, in vivo cytokine and/or CAL is covered by the present invention, so long as the initial dose of cells is capable of eliciting a beneficial GVT response. This dose of cells corresponds to a number of HLA-compatible allogeneic

peripheral blood lymphocytes that elicits a host response beyond mere immunization to a second dose of cells from the same or similar donor. Generally, this number corresponds to at least about 10^7 allogeneic peripheral blood lymphocytes/kg. However, it is possible that a lower dose of cells, e.g., about 10^5 cells/kg, could be used if, for example, CAL were used for the initial infusion.

Between the Allo-CMI/CCI treatments or at the conclusion of an Allo-CMI/CCI regimen, the patient may be monitored for levels of malignant cells, i.e., for evidence of minimal residual disease. Such monitoring may comprise patient follow-up for clinical signs of relapse. The monitoring may also include, where appropriate, various molecular or cellular assays to detect or quantify any residual malignant cells. For example, in cases of sex-mismatched donors and recipients, residual host-derived cells may be detected through use of appropriate sex markers such as Y chromosome-specific nucleic acid primers or probes. In cases of single HLA locus mismatches between donors and recipients, residual host cells may be documented by polymerase chain reaction (PCR) analysis of Class I or Class II loci that differ between the donor and recipient. Alternatively, appropriate molecular markers specific for tumor cells can be employed. For example, nucleic acid primers

and/or probes specific for the bcr/abl translocation in chronic myelogenous leukemia, for other oncogenes active in various tumors, for inactivated tumor suppressor genes, other tumor-specific genes, or any other assay reagents known to be specific for tumor cells, may be employed. Any of these or functionally comparable procedures may be used to monitor the patient for evidence of residual malignant cells.

Under normal circumstances, recipients of autologous or allogeneic bone marrow grafts receive only irradiated blood products when such products are required by the patient. These products are irradiated in order to avoid the possibility of engraftment of immunocompetent T-lymphocytes derived from the donor's blood product (e.g., platelets or red blood cells). In most institutions, irradiated blood products are also used for patients receiving high dose conventional chemotherapy without transplant, e.g., blood products given following induction of remission in leukemia and lymphoma patients. Obviously, the chances for engraftment of immunocompetent T-lymphocytes from otherwise mismatched blood products is relatively small under normal circumstances. However, if immunosuppression is sufficient to permit engraftment, GVHD can be "stormy" and lethal.

In the methods of the present invention, non-irradiated donor-type lymphocytes are used intentionally for induction of graft-versus-malignant cell effects. The methods are structured to produce transient engraftment, so as to induce graft-versus-malignant cell effects that may be accompanied by mild GVHD.

Since the donor cells used in the Allo-CMI and Allo-CCI procedures are HLA-compatible with the recipient, chances of engraftment are better than if the donor's cells were not functionally matched with the patient's major histocompatibility complex. Moreover, the chance of immediate rejection on the one hand and lethal GVHD on the other hand are relatively small because of the HLA compatibility. As such, the Allo-CMI/CCI protocols of the present invention provide the possibility for transient engraftment of donor's PBL with effective GVT and with a minimal chance for induction of severe GVHD.

The invention will be further understood with reference to the following illustrative embodiments, which are purely exemplary, and should not be taken as limiting the true scope of the present invention as described in the claims.

EXAMPLE 1

Syngeneic BMT (SBMT) in Mice Followed by Allo-CMI or Allo-CCI

I. METHODS

A. Mice. BALB/c (BALB), and C57BL/6 (C57), (BALB/c x C57BL/6)F1 (F1) mice, 2-6 months old, were purchased from the breeding colony of the Hebrew University-Hadassah Medical School, Jerusalem. Mice were kept under standard conditions, with acidic water (pH 2.7) and no special protective measures. Mice were given 0.5% neomycin sulfate in their drinking water for 2 weeks post-transplantation.

rate of 60 cGy/min. Alternatively, mice were conditioned with freshly dissolved cyclophosphamide (CY) (300 mg/kg) (Taro, Israel) given intraperitoneally (IP). Twenty-four hours later, mice received 10^7 syngeneic marrow cells via the lateral tail vein.

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E. Preparation of Bone Marrow Cells (BMC). BMC were obtained from the femura, tibiae and humera of syngeneic mice. Mononuclear cells containing 10^7 BMC in 0.25 ml Hank's medium were injected into the lateral tail vein of recipients 24 hours post-radiation.

F. Purging Procedure. Cells were resuspended at a concentration of 20×10^6 cells/ml in Hank's medium containing 4% human serum albumin. ASTA-Z was then added to a final concentration of 100 μ g/ml. Both untreated control cells and ASTA-Z treated BMC were incubated at 37°C for 30 minutes, washed twice in Hank's medium and counted. Purged or unpurged BMC (4×10^6) were injected into BALB mice conditioned with CY.

G. Recombinant Human Interleukin-2 (rhIL2). rhIL2 provided as 1 mg Proleukin (3×10^6 Cetus Units, equivalent to 18×10^6 International Units) was kindly supplied by Dr. S.L. Aukerman, Cetus/Chiron, CA, USA. rhIL2 was initially diluted with water for injection and subsequently rediluted with dextrose 5%. International units (IU) are used throughout the remainder of the present application.

H. Activation of BMC by rhIL2. BMC were cultured in 225 cm³ flasks (Corning 25160-225, Corning Glass, Corning NY) in RPMI 1640 medium (Beit Haemek, Israel) containing L-glutamine, non-essential amino acids, pyruvate, 10%

5 bovine calf serum (BCS) and rhIL2 (6,000 IU/ml) for 4 days in a humidified incubator with 5% CO₂ at 37°C. Following harvesting, viability was determined by the trypan blue exclusion method.

I. Simulation of Minimal Residual Disease Following Syngeneic Bone Marrow Transplantation. In order to simulate minimal residual disease (MRD) quantitatively, 10⁵ BCL1 cells were added to the marrow inoculum during syngeneic bone marrow transplantation (SBMT), prior to immunotherapy.

J. Immunotherapy by Immunocompetent Allogeneic Lymphocytes. Allogeneic cell-mediated immunotherapy (Allo-CMI) consisted of adoptive transfer of immunocompetent allogeneic lymphocytes (PBL or a mixture of donor spleen and lymph node cells) as detailed in the results for each experiment, below. Allogeneic cell-mediated cytokine-activated immunotherapy (Allo-CCI) consisted of adoptive transfer of allogeneic lymphocytes pre-activated in vitro with rhIL2
20 (allogeneic "CAL") In some experiments allogeneic lymphocyte infusion was followed by subsequent in vivo activation with rhIL2, by additional Allo-CMI with in vivo rhIL-2, or by additional Allo-CCI with in vivo rhIL-2, respectively.

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K. Detection of Residual Clonogenic BCL1 by Adoptive Transfer Experiments. In order to determine whether or not residual BCL1 cells were present after various treatments, 10^5 spleen cells obtained from treated mice were adoptively transferred to untreated secondary syngeneic (BALB) recipients. Absence of leukemia (≥ 100 days) in secondary recipients was indicative of elimination of BCL1 since as few as 1-10 cells were previously shown to cause disease.

L. Statistical Analysis. The significance of differences between treated and untreated mice was calculated by the independent statistical t-test.

II. RESULTS

A. Induction of Allo-CMI and Allo-CCI Effects. F1 mice were lethally irradiated (750 cGy) and transplanted with 10^7 syngeneic BMC. Following inoculation of 10^5 BCL1 cells to simulate MRD, varying numbers of C57 PBL were administered intravenously to induce GVL-like effects through allo-CMI. In order to detect the efficacy of allo-CMI in eradicating residual BCL1 cells, aliquots of 10^5 spleen cells pooled from 2-3 experimental mice were adoptively transferred to secondary normal BALB recipients, one or two weeks post-SBMT.

Figure 1 summarizes results obtained from three different experiments in a total of 120 mice. Injection of $20-30 \times 10^6$ PBL, obtained from C57 mice to induce allo-CMI after SBMT in F1 recipients, effectively eliminated residual BCL1 cells, as

none of 40 secondary adoptive BALB recipients developed leukemia (>180 days). In contrast, leukemia developed in all 20 secondary BALB recipients inoculated with 10^5 spleen cells obtained from F1 recipients that had received $20-30 \times 10^6$ PBL from syngeneic donors post-SBMT. Addition of rhIL2 (12×10^4 IU $\times 2$ /day for 5 days IP)

5 post-transplant did not improve the disease-free survival of secondary recipients of 10^5 spleen cells (obtained from similarly treated F1 mice) since all 20 secondary recipient BALB mice developed leukemia (Figure 1). Addition of rhIL2 in vivo at the same dose to recipients of 20×10^6 allogeneic PBL for further in vivo activation of effector cells did not induce measurable additional GVL effects since all 40 secondary BALB recipients remained disease free (>180 days) (Figure 1).

B. Quantitative Effect of the Number of Effector Cells on the Efficacy of Allo-CMI. Anti-leukemic effects mediated by allo-CMI were cell-dose dependent. As shown in Figure 2, all SBMT recipients injected with 30×10^6 C57 spleen cells completely resisted the development of leukemia following inoculation of 10^5 BCL1 cells. Injection of 10×10^6 allogeneic spleen cells together with 10^5 BCL1 cells induced effective allo-CMI in 70% of the secondary adoptive recipients. However, reduction of allo-CMI inducing C57 spleen cells to 3×10^6 or 1×10^6 failed to eliminate residual BCL1 cells and all secondary adoptive recipients developed leukemia (Figure 2).

20 C. Induction of Allo-CMI and Allo-CMI/IL-2 Effects Following Transplantation with ASTA-Z-Purged BMC. The feasibility of induction of allo-CMI

was investigated by conditioning with high-dose CY followed by rescue of recipients with ASTA-Z-purged syngeneic BMC. BALB recipients received high-dose CY (300 mg/kg IP) and were injected 24 hours later with 10^3 BCL1 cells to simulate MRD. One day later, all mice received intravenously 4×10^6 ASTA-Z treated syngeneic BMC.

5 Mice were divided into 3 experimental groups: the first group (6 mice) received intravenously a mixture of allogeneic C57 spleen and lymph node cells (20×10^6 cells) for induction of allo-CMI; the second group (6 mice) received identical cell therapy with additional in vivo potentiation of GVL by rhIL2 treatment (12×10^4 IU x3/day for 3 days, IP); the third group (7 mice) received a mixture of syngeneic spleen and lymph node cells, with an identical in vivo rhIL2 treatment. One week later, aliquots of 10^5 cells from a pool of 2-3 spleens obtained from each experimental group were adoptively transferred to secondary BALB mice.

As shown in Figure 3, all mice inoculated with spleen cells from control mice given 10^3 BCL1 cells, or mice given syngeneic BALB lymphocytes with in vivo rhIL2, developed leukemia and died within 40 and 60 days, respectively. Likewise, secondary recipients of 10^5 spleen cells obtained from mice that were treated with allo-CMI, using allogeneic C57 cells alone, showed no measurable GVL effects since all recipients developed leukemia. In contrast, addition of rhIL2 in vivo following administration of C57 spleen and lymph node cell mixtures induced
20 substantial anti-leukemic effects and 50% of the secondary adoptive recipient mice remained leukemia-free for >125 days (Figure 3).

D. Enhancement of Immunotherapeutic Effect by In vitro Activation of Allogeneic Lymphocytes with rhIL2. The following experiment was designed to test for potential enhancements in efficacy of treatment by in vitro pre-activation of allogeneic effector cells with rhIL2. Lethally irradiated (750 cGy TBI) F1 mice were

5 infused with 30×10^6 C57 BMC pre-activated in vitro for 4 days with rhIL2. BMC were mixed with 10^5 BCL1 cells to simulate MRD. Results of 3 separate sets of experiments gave similar results and therefore the data were pooled (Figure 4).

All F1 recipients were divided into two groups. The first group of 33 mice received no additional treatment. Mice in the second group (25 mice) were injected with rhIL2 (12×10^4 IU x2/day for 5 days, IP) in an attempt to further increase efficacy of cell therapy by continuous activation of rhIL2-dependent effector cells in vivo. Aliquots of 10^5 cells obtained from a pool of spleen cells prepared from mice of either experimental group were adoptively transferred to secondary BALB recipients. As shown in Figure 4, 10 of 33 secondary recipients of spleen cells obtained from the first experimental group remained disease-free for >150 days. Additional in vivo rhIL2 therapy in the second experimental group further improved the Allo-CCI effects, as 19 of 25 secondary recipients remained disease-free for an observation period >150 days ($p=0.05$) (Figure 4).

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EXAMPLE 2

Autologous Stem Cell Transplantation (ASCT) in Humans

Followed by Allo-CMI and/or Allo CCI

~~5 I. PATIENT TREATMENT PROTOCOLS~~

Patient No. 1. This female patient was diagnosed with acute myelogenous leukemia (AML), French American British (FAB) classification M4, and was in first complete remission (i.e., no evidence of disease) at the time of autologous stem cell transplantation (ASCT). The patient was 41 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of Busulfan, 4 mg/kg, days 6 through 9 pre-ASCT (days -9 to -6), as well as Cytosan (cyclophosphamide), 50 mg/kg, days -5 to -2, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and cytosine arabinoside (Ara-C), 25 mg intrathecally.

Prior to ASCT, the autologous cells to be infused were purged by treatment with Mafosfamide (ASTA-Z). ASTA-Z was provided by Drs. M. Peukert and H. Sindermann (Astawerke, Bielefeld, Germany) as a lyophilized powder and was freshly dissolved in saline before use. Autologous cells were resuspended at a concentration of 20×10^6 cells/ml in Hank's medium containing 4% human serum albumin. ASTA-Z was then added to a final concentration of 100ug/ml and the cells were incubated in the ASTA-Z at 37° C for 30 min. After this, the cells were washed twice in Hank's medium and counted. Cells were cryopreserved and kept in liquid

nitrogen until used. ASCT consisted of 2.5×10^8 nucleated bone marrow cells/kg, infused intravenously (IV) on day 0.

On day 1 following ASCT (day +1), the patient received 10^4 T cells/kg of peripheral blood lymphocytes (PBL) from an HLA-matched donor. On days +8, +22, +29 and +36 she received PBL from the same donor at an equivalent dose of 10^5 , 10^5 , 10^6 , and 10^6 T cells/kg, respectively. On day +47 she received PBL from the same donor at an equivalent dose of 10^7 T cells/kg. The patient showed no evidence of GVHD.

Patient No. 2. This female patient was diagnosed with AML, FAB M5, and was in first complete remission at the time of ASCT. The patient was 42 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of Busulfan, 4 mg/kg, (days -9 to -6), as well as Cytosan, 50 mg/kg, days -5 to -2, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally.

Prior to ASCT, the autologous bone marrow cells to be infused were purged by treatment with ASTA-Z. Autologous cells were resuspended at a concentration of 20×10^6 cells/ml in Hank's medium containing 4% human serum albumin. ASTA-Z was then added to a final concentration of 100ug/ml and the cells were incubated in the ASTA-Z at 37°C for 30 min. After this, the cells were washed twice in Hank's medium, counted and cryopreserved. ASCT consisted of 1×10^8 nucleated bone marrow cells/kg, infused IV on day 0.

On day +1, the patient received 10^4 T cells/kg of PBL from an HLA-matched donor. On days +8, +18 and +26 she received PBL from the same donor at an equivalent dose of 10^5 , 10^6 , and 10^7 T cells/kg, respectively. On day +80 she received PBL from the same donor at an equivalent dose of 10^7 T cells/kg, with 3×10^6 IU of rhIL-2/ m^2 /day for 3 days, by subcutaneous injection beginning on day +80. The patient showed no evidence of GVHD.

Patient No. 3. This female patient was diagnosed with AML, FAB M3, and was in first complete remission at the time of ASCT. The patient was 32 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of Busulfan, 4 mg/kg, (days -9 to -6), as well as Cytosan, 50 mg/kg, days -5 to -2, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally. ASCT consisted of non-purged bone marrow cells, and 0.79×10^8 nucleated cells/kg were infused IV on day 0. On day +1, the patient received PBL from an HLA-matched donor at an equivalent dose of 10^7 T cells/kg. On day +1, she also received 6×10^6 IU of rhIL-2/ m^2 by subcutaneous injection. No GVHD was observed.

Patient No. 4. This male patient was diagnosed with AML, FAB M2, and was in first complete remission at the time of ASCT. The patient was 23 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of Busulfan 4 mg/kg, days -9 to -6, Cytosan 60 mg/kg, days -3 to -2, Thiotepea 5 mg/kg/day, days -5

to -4, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally. ASCT consisted of non-purged 1.37×10^8 nucleated bone marrow cells/kg, infused IV on day 0. On day +1, the patient received PBL from an HLA-matched donor at an equivalent dose of 10^7 T cells/kg. On day +1, he also received 6×10^6 IU of rhIL-2/m² by subcutaneous injection. GVHD was suspected (Grade I) in the skin.

Patient No. 5. This male patient was diagnosed with chronic myelogenous leukemia (CML) in chronic phase. The original CML karyotype was positive for the Philadelphia chromosome (Ph+). The patient was in chronic phase (CP) and was Ph- at the time of ASCT. The patient was 51 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of total body irradiation (TBI) 200 cGY/day, days -5 to -3, and Cytosan 60mg/kg, days -2 to -1. ASCT consisted of non-purged 0.5×10^8 nucleated bone marrow cells/kg, infused IV on day 0.

On day +71, as soon as blood counts had stabilized (WBC: 4,200/mm³; Hb: 11.5 g%; platelets: 133,000/mm³), the patient received 3×10^7 T cells/kg of PBL from an HLA-matched brother. No GVHD was observed; hence, the allo-CMI regimen was escalated. On day +107, the patient received 4.6×10^7 T cells/kg of PBL from the same donor. Starting on day +107, he also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD was observed. On day +240, after the Ph+ karyotype had reappeared, the patient received 4.95×10^7 cells/kg of cytokine-activated lymphocytes ("CAL") from the same donor. Starting on day

+240, he also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. The CAL were produced by exposing the donor's PBL to 6,000 IU/ml rhIL-2 for four days in culture.

5 Patient No. 6. This female patient was diagnosed with AML, FAB M2, and was in first complete remission at the time of ASCT. The patient was 50 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of Busulfan 4 mg/kg, days -9 to -6, Cytosan 60 mg/kg, days -3 to -2, Thiotepe 5 mg/kg/day, days -5 to -4, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally. ASCT consisted of non-purged 0.64×10^8 nucleated bone marrow cells/kg infused IV on day 0. On day +58, as soon as blood counts had stabilized (WBC: 4,400/mm³; Hb: 9.5 g%; platelets: 66,000/mm³), the patient received 5×10^7 T cells/kg of PBL from an HLA-matched sister. On day +86 the patient received a second dose of 6.1×10^7 T cells/kg of PBL from the HLA-matched sister. Starting on day +86, she also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD was observed.

20 Patient No. 7. This male patient was diagnosed with CML. The original CML karyotype was Ph+. The patient was in chronic phase (CP) and 50% of his marrow cells were Ph+ (i.e., the patient was Ph+) at the time of ASCT. The patient was 47 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of TBI 200 cGY/day, days -5 to -3, and Cytosan 60mg/kg, days -2 to -1. ASCT consisted of

non-purged 0.98×10^8 nucleated bone marrow cells/kg, infused IV on day 0. On day +55, as soon as blood counts had stabilized (WBC: 6,900/mm³; Hb: 12.0 g%; platelets: 248,000/mm³), the patient received 4×10^7 T cells/kg of PBL from an HLA-matched sister. No GVHD developed. On day +77, the patient received 2.8×10^7 cells/kg of PBL from the HLA-matched sister. Starting on day +77, he also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD was observed.

Patient No. 8. This male patient was diagnosed with non-Hodgkin's lymphoma (NHL), Burkitt-like, and was in a second partial remission at the time of ASCT. As used herein, the term "partial remission" indicates at least a 50% response (i.e., at least a 50% reduction of lymphoma cell mass) but with continued evidence of disease. The patient was 36 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of etoposide, 200 mg/m²/day, days -6 to -3, thiotepa, 40 mg/m²/day, days -5 to -2, Ara-C, 200 mg/m²/day, days -4 to -1, Cytosan, 60 mg/kg/day, day -3, and melphalan, 60 mg/m²/day, days -2 to -1.

ASCT consisted of 0.74×10^8 /kg viable bone marrow nucleated cells plus 2.36×10^8 /kg viable peripheral blood stem cells. Subcutaneous GM-CSF, 5ug/kg/day, was administered from day +1 to day +18. Prior to ASCT, the autologous cells were purged with Dynal magnetic beads coated with anti-CD19 for elimination of residual lymphoma cells.

On day +90, the patient received 5×10^7 cells/kg of PBL from an HLA-matched brother. The patient showed no signs of GVHD following this first cell

infusion. Polymerase chain reaction (PCR) analysis using two VNTR loci (VNTR = Variable Number of Tandem Repeats) revealed no evidence of circulating donor-specific cells. On day +124, the patient received 5×10^7 cells/kg of PBL from the same donor. This was followed by three days outpatient treatment with 6×10^6 IU of rhIL-

5 2/m²/day, by subcutaneous injection, beginning on day +124.

Fifty days later (day +174) the patient developed pancytopenia, and bone marrow biopsy revealed severe hypocellular marrow with increased numbers of large granular lymphocytes and plasma cells. Lymphocytes with a similar morphology were found in the blood. Repeated PCR using two different VNTR loci showed partial engraftment of donor cells on day +191 and 100% engraftment of donor cells on day +211. An allogeneic BMT was then performed by infusing the patient with 3.4×10^8 /kg of the donor's marrow cells; no post-transplant anti-GVHD prophylaxis was administered. The patient had rapid three-lineage engraftment with normal platelet counts after 14 days and normal hemoglobin after 26 days. WBC normalized after 10 days with 70% neutrophils, 5% monocytes and 25% lymphocytes. Large granular lymphocytes disappeared from the blood. On day 14 following allogeneic BMT, the patient showed minimal signs of acute GVHD with involvement of skin and oral cavity. There was no intestinal or liver involvement. Since then the patient has continued to experience grade I/II mucocutaneous

20 GVHD, partially controlled with steroids and cyclosporin A.

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mg/m²/day, days -5 to -2, Ara-C, 200 mg/m²/day, days -4 to -1, Cytosan, 60 mg/kg/day, day -3, and melphalan, 60 mg/m²/day, days -2 to -1.

ASCT consisted of 0.94×10^8 non-purged nucleated bone marrow cells/kg plus 3.9×10^7 peripheral blood stem cells mobilized by G-CSF prior to

5 collection with the CS 3000® Plus blood cell separator (Baxter Healthcare Corporation, Fenwal System Catalogue No. 4R4538). Cells were infused IV on day 0.

At week 10 post-ASCT, as soon as blood counts had stabilized (WBC: 4,300/mm³; Hb: 11.2 g%; platelets: 116,000/mm³), the patient received 3×10^7 T cells/kg of PBL from an HLA-matched brother. At week 15 post-ASCT, the patient received 4.1×10^7 T cells/kg of PBL from the same donor. Starting at week 15, on the same day as the administration of the second Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. At week 23 post-ASCT, the patient received 5×10^7 CAL/kg from the HLA-matched brother. The CAL cells were produced by exposing the donor's PBL to 6,000 IU/ml rhIL-2 for four days in culture. Starting at week 23, on the same day as the administration of the Allo-CCI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD was observed.

Patient No. 11. This female patient was diagnosed with NHL, immunoblastic, and was in a second complete remission at the time of ASCT. The patient was 21 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of etoposide, 200 mg/m²/day, days -6 to -3; thiotepea, 40

mg/m²/day, days -5 to -2; Ara-C, 200 mg/m²/day, days -4 to -1; Cytosan, 60 mg/kg/day, day -3; and melphalan, 60 mg/m²/day, days -2 to -1.

ASCT consisted of 3.82×10^8 non-purged bone marrow cells/kg plus 1.29×10^8 peripheral blood stem cells mobilized with G-CSF prior to collection with

5 the CS 3000® Plus blood cell separator. Cells were infused IV on day 0.

At week 10 post-ASCT, the patient received 5×10^7 T cells/kg of PBL from an HLA-matched sister. At week 15 post-ASCT, the patient received a second infusion of 5×10^7 T cells/kg of PBL from the HLA-matched sister. At week 19 post-ASCT, the patient received a third infusion of 5×10^7 T cells/kg of PBL from the same donor. No GVHD was observed, but the patient did not accept the suggestion that she receive in vivo rhIL2. At week 30 post-ASCT, the patient received a fourth infusion of 5×10^7 T cells/kg of PBL from the HLA-matched sister. Starting at week 23, on the same day as the administration of the fourth Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD has been observed to date.

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Patient No. 12. This female patient was diagnosed with NHL, diffuse large cell, and was in a condition of relapse at the time of ASCT. The patient was 21 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of
20 etoposide, 200 mg/m²/day, days -6 to -3; thiotepa, 40 mg/m²/day, days -5 to -2; Ara-C, 200 mg/m²/day, days -4 to -1; Cytosan, 60 mg/kg/day, day -3; and melphalan, 60 mg/m²/day, days -2 to -1.

ASCT consisted of 1.8×10^8 non-purged mononuclear bone marrow cells/kg, infused IV on day 0.

At week 6 post-ASCT, as soon as blood counts had stabilized (WBC: 4,400/mm³; Hb: 11.7 g%; platelets: 150,000/mm³), the patient received 5×10^7 T cells/kg of PBL from a single locus-mismatched sister. At week 10 post-ASCT, the patient received a second infusion of 5×10^7 T cells/kg of PBL from the same donor. Starting at week 10, on the same day as the administration of the second Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD was observed.

Patient No. 13. This male patient was diagnosed with AML, FAB M5, and was in first complete remission at the time of ASCT. The patient was 46 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of Busulfan 4 mg/kg, days -9 to -6; Cytosan 60 mg/kg, days -3 to -2; Thiotepe 5 mg/kg/day, days -5 to -4; Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C; 25 mg intrathecally. ASCT consisted of 1.39×10^8 non-purged bone marrow cells infused IV on day 0. At week 11, the patient received 3.86×10^7 T cells/kg of PBL from an HLA-matched brother. No GVHD developed.

20 Patient No. 14. This female patient was diagnosed with AML, FAB M3, and was in second partial remission at the time of ASCT. The patient was 12 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of Busulfan

4 mg/kg, days -9 to -6; Cytosan 60 mg/kg, days -3 to -2; Mitoxantrone 6 mg/m²/day, days -5 to -4; Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally. ASCT consisted of 1.14×10^8 non-purged marrow cells infused IV on day 0. At week 16, the patient received 2×10^7 T cells/kg

5 of single locus-mismatched PBL from her mother. At week 20, the patient received 1000 mg/m² Cytosan in order to improve the efficacy and temporal engraftment of a second infusion of the donor's PBL. Twenty four hours later, the patient received 5×10^7 T cells/kg from the same donor. Starting on the same day as the administration of the second Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD has been noted thus far.

Patient No. 15. This male patient was diagnosed with AML, FAB M5, and was in first complete remission at the time of ASCT. The patient was 6 1/2 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of Busulfan 4 mg/kg, days -9 to -6; Cytosan 60 mg/kg, days -3 to -2; Thiotepa 5 mg/kg/day, days -5 to -4; Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally. ASCT consisted of 2.7×10^8 non-purged marrow cells infused IV on day 0. At week 14, the patient received 5×10^7 T cells/kg of single

20 locus-mismatched PBL from his father. No GVHD has developed, and the patient is scheduled to receive a second dose of allo-CMI plus in vivo rhIL2.

Patient No. 16. This male patient was diagnosed with NHL, mixed large and small cell, in the lymph nodes, in February of 1993. There was also heavy bone marrow involvement. He received chemotherapy (12 courses of MACOP-B), but relapsed in the lymph nodes and bone marrow in October of 1993. He underwent

5 additional chemotherapy and was in a second partial remission at the time of ASCT, with recurrence in the marrow. The patient was 45 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen consisting of etoposide, 200 mg/m²/day, days -6 to -3; thiotepa, 40 mg/m²/day, days -5 to -2; Ara-C, 200 mg/m²/day, days -4 to -1; Cytosan, 60 mg/kg/day, day -3; and melphalan, 60 mg/m²/day, days -2 to -1.

ASCT consisted of 2.15×10^8 non-purged G-CSF-mobilized peripheral blood stem cells, infused IV on day 0.

At week 7 post-ASCT, the patient received 3×10^7 T cells/kg of PBL from an HLA-matched sister. At week 11 post-ASCT, the patient received a second infusion of 3×10^7 T cells/kg of PBL from the same donor. Starting at week 11, on the same day as the administration of the second Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD has been observed to date.

20 Patient No. 17. This female patient was diagnosed with metastatic breast cancer, with metastases to the liver and spine. She underwent a right partial mastectomy with axillary lymph node dissection showing involvement of 18/35

lymph nodes. The patient was 43 years old at the time of ASCT. Prior to ASCT, she received a conditioning regimen of carboplatin, 200 mg/m²/day, days -7 to -4; thiotepa, 60 mg/m²/day, days -6 to -4; etoposide, 200 mg/m²/day, days -5 to -3; and melphalan, 60 mg/m²/day, days -4 to -3.

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ASCT consisted of 3.7×10^8 peripheral blood stem cells/kg, infused IV on day 0. The stem cells were collected after mobilization with G-CSF (7.5 mg/kg/day for 5 days) using three collections with the CS 3000® Plus blood cell separator. Following ASCT, the patient recovered with no complications and was discharged on day 20 post-ASCT.

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A fully HLA-matched (A, B, DR and mixed lymphocyte reaction (MLR)-negative) sibling was chosen as donor of PBL for Allo-CMI. At week 8 post-ASCT, as soon as the patient achieved a stable clinical condition (WBC: 2,700/mm³; Hb: 9.1 g%; platelets: 56,000/mm³), the patient received 5×10^7 T cells/kg of PBL from the HLA-matched sibling. Starting at week 8, on the same day as the administration of the first Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD has been observed to date.

20 Patient No. 18. This male patient was diagnosed with metastatic breast cancer, with metastases to the bone marrow, sternum and vertebrae T₄ to T₇. He underwent lumpectomy with axillary lymph node dissection showing involvement of 16/18 lymph nodes. The patient was 36 years old at the time of ASCT. Prior to ASCT, he received a conditioning regimen of carboplatin, 200 mg/m²/day, days -7 to

-4; thiotepa, 60 mg/m²/day, days -6 to -4; etoposide, 200 mg/m²/day, days -5 to -3; and melphalan, 60 mg/m²/day, days -4 to -3.

ASCT consisted of 1.81×10^8 peripheral blood stem cells/kg, infused IV on day 0. The stem cells were collected after mobilization with G-CSF

5 (7.5 mg/kg/day for 5 days) using three collections with the CS 3000® Plus blood cell separator. Following ASCT, the patient recovered with no complications and was discharged.

A fully HLA-matched (A, B, DR and mixed lymphocyte reaction (MLR)-negative) brother was chosen as donor of PBL for Allo-CMI. At week 5 post-ASCT, as soon as the patient achieved a stable clinical condition (WBC: 11,000/mm³; Hb: 11.5 g%; platelets: 201,000/mm³), the patient received 2.3×10^7 T cells/kg of PBL from the HLA-matched brother. Starting at week 5, on the same day as the administration of the first Allo-CMI treatment, the patient also received 6×10^6 IU of rhIL-2/m²/day for 3 days, by subcutaneous injection. No GVHD has been observed to date.

Portions of the above-described patient treatment protocols are summarized in Table 1, below. Patients are grouped according to disease (AML, CML, NHL and breast cancer).

TABLE 1a

AML:

	Patient No.	Status Pre-ASCT	Cond. Regimen	Type ASCT	Allo Cells (T Cells/Kg)
5	1	1CR	BU/CY	ABMT: Purged	PBL $10^4 - 10^7$ (day +1 to + 47)
	2	1CR	BU/CY	ABMT: Purged	PBL $10^4 - 10^7$ (day +1 to + 80)
10	3	1CR	BU/CY	ABMT: Non-Purged	PBL 10^7 + IL2 (day +1)
	4	1CR	BU/CY + TT	ABMT: Non-Purged	PBL 10^7 + IL2 (day +1)
	6	1CR	BU/CY + TT	ABMT: Non-Purged	1. PBL 2. PBL + IL2
15	13	1CR	BU/CY + TT	ABMT: Non-Purged	1. PBL
	14	2PR	BU/CY + MX	ABMT: Non-Purged	1. PBL
	15	1CR	BU/CY + TT	ABMT: Non-Purged	2. Cytosan/PBL + IL2 1. PBL

CML:

Patient No.	Status Pre-ASCT	Cond. Regimen	Type ASCT	Allo Cells (T Cells/Kg)
5	CP(Ph-)	CY/TBI	ABMT: Non-Purged	1. PBL 2. PBL + IL2 3. CAL + IL2
7	CP(Ph+)	CY/TBI	ABMT: Non-Purged	1. PBL 2. PBL + IL2

NHL:

Patient No.	Status Pre-ASCT	Cond. Regimen	Type ASCT	Allo Cells (T Cells/Kg)
8	2PR	ETACM	ABMT + PBSC: Purged	1. PBL 2. PBL + IL2 3. Allogeneic BMT (due to marrow aplasia)
9	2PR	ETACM	ABMT : Purged	1. PBL 2. PBL + IL2 3. CAL + IL2
10	2CR	ETACM	ABMT + PBSC: Non-Purged	1. PBL 2. PBL + IL2 3. CAL + IL2
11	2CR	ETACM	ABMT + PBSC: Non-Purged	1. PBL 2. PBL 3. PBL
12	Relapse	ETACM	ABMT : Non-Purged	4. PBL + IL2 1. PBL 2. PBL + IL2
16	2PR	ETACM	ABMT + PBSC: Non-Purged	1. PBL 2. PBL + IL2

BREAST CANCER:

Patient No.	Status Pre-ASCT	Cond. Regimen	Type ASCT	Allo Cells (T Cells/Kg)
17	Metastatic	CTEM	PBSC: Non-Purged	1. PBL + IL2
18	Metastatic	CTEM	PBSC: Non-Purged	1. PBL + IL2

^a Terms used in the Table are defined as follows:

1CR, 2CR: First or second complete remission, respectively.

2PR: Second partial remission

CP(Ph⁻): Chronic phase, no cytogenetic evidence of Philadelphia chromosome.

CP(Ph⁺): Chronic phase, cytogenetic evidence for presence of Philadelphia chromosome.

BU/CY: Conditioning regimen of Busulfan, 4 mg/kg, days 6 through 9 pre-ASCT (days -9 to -6), as well as Cytosan (cyclophosphamide), 50 mg/kg, days -5 to -2, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and cytosine arabinoside (Ara-C), 25 mg intrathecally.

BU/CY + TT: Conditioning regimen of Busulfan 4 mg/kg, days -9 to -6, Cytosan 60 mg/kg, days -3 to -2, Thiotepea 5 mg/kg/day, days -5 to -4, Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally.

BU/CY + MX: Conditioning regimen of Busulfan 4 mg/kg, days -9 to -6; Cytosan 60 mg/kg, days -3 to -2; Mitoxantrone 6 mg/m²/day, days -5 to -4; Cotrimoxazol, 10 mg/kg, days -10 to -2, Allopurinol, 300 mg/kg, days -10 to -1 and Ara-C, 25 mg intrathecally.

CY/TBI: Conditioning regimen of total body irradiation (TBI) 200 cGY/day, days -5 to -3, and Cytosan 60mg/kg, days -2 to -1.

- 5 ETACM: Conditioning regimen of etoposide, 200 mg/m²/day, days -6 to -3; thiotepa, 40 mg/m²/day, days -5 to -2; Ara-C, 200 mg/m²/day, days -4 to -1; Cytosan, 60 mg/kg/day, day -3; and melphalan, 60 mg/m²/day, days -2 to -1.

- 10 ~~CTEM: Conditioning regimen of carboplatin, 200 mg/m²/day, days -7 to -4; thiotepa, 60 mg/m²/day, days -6 to -4; etoposide, 200 mg/m²/day, days -5 to -3; and melphalan, 60 mg/m²/day, days -4 to -3.~~

ABMT: Infusion of stem cells extracted directly from the bone marrow.

PBSC: Infusion of stem cells taken from peripheral blood following mobilization from the bone marrow.

- 15 PBL, CAL: Without further numerical designation, PBL (peripheral blood lymphocytes) and CAL (cytokine-activated lymphocytes) refer to one infusion of $\geq 10^7$ cells/kg of patient body weight. For patient No.'s 5-18, the first infusion of allogeneic cells was done only after attainment of partial hematopoiesis reconstitution.

- 20 Cytosan/PBL: Cyclophosphamide (Cytosan) administered prior to infusion of $\geq 10^7$ cells/kg of PBL.

II. RESULTS

The above-described patients have been followed for various lengths of time. The disease status of the patients is summarized below:

Patient No. 1 relapsed after 6 months and died at 9 months after ASCT.

Patient No. 2 relapsed after 12 months and died at 19 months after ASCT.

Patient No. 3 is alive and well over 34 months after ASCT.

Patient No. 4 is alive and well over 24 months after ASCT.

Patient No. 5 is alive and well over 23 months after ASCT, showing no signs of the Philadelphia chromosome (Ph-) by cytogenetic analysis and being negative by PCR for the bcr/abl translocation characteristic of the Philadelphia chromosome.

- 35 The patient is receiving 9×10^6 units of IFN α (Roferon A, S.C.) every day.

Patient No. 6 is alive and well over 23 months after ASCT with no evidence of disease.

Patient No. 7 is alive and well over 21 months post-ASCT. Currently, the patient is on IFN α treatment, and is hematologically normal with no clinical sign of

5 disease.

Patient No. 8, currently at *over 29 months* ~~nearly 2 years~~ after initial ASCT and over one year following allogeneic BMT, is alive and well with no evidence of lymphoma. The patient has mild cutaneous chronic GVHD with Karnofsky score of 100%

Patient No. 9 was alive and well until 18 months after ASCT. At 19 months he has lymphadenopathy and is being evaluated for relapse.

Patient No. 10 is alive and well over 15 months after ASCT with no evidence of disease.

Patient No. 11 is alive and well over 13 months after ASCT with no evidence of disease.

Patient No. 12 underwent relapse at 3 months post-ASCT and died at 5 months post-ASCT.

Patient No. 13 underwent relapse at 4 months post-ASCT, before receiving allo-CMI with rhIL2. He is alive and further immunotherapy is planned.

Patient No. 14 is alive and well with no evidence of disease over 5 months post-ASCT.

Patient No. 15 is alive and well with no evidence of disease over 4 months post-ASCT.

Patient No. 16 is alive and well with no evidence of disease 3 months post-ASCT.

Patient No. 17 is alive and well with no evidence of disease 2 months post-ASCT.

5 Patient No. 18 is alive and well with no evidence of disease 1.5 months post-ASCT.

The results reported above indicate that Allo-CMI and Allo-CCI may be the most rational and practical approaches for eradication of residual malignant cells. GVT effects induced by administration of allogeneic lymphocytes may be further enhanced by administration in vivo of rhIL2.

EXAMPLE 3

Utility of Cytokines Other Than IL2

Cytokines used in the following experiments were obtained as follows:

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- (1) rhIL2 was provided by Dr. C.R. Franks (EuroCetus BY, Amsterdam, The Netherlands) as a lyophilized powder in 1 mg vials containing 18×10^6 international units (IU).
 - (2) Recombinant interferon-gamma (rIFN γ) was provided by Roussel Uclaf (Romainville, France) as a lyophilized powder containing 2×10^7 U/mg.
 - (3) Recombinant human IL-6 (rIL-6) was kindly provided by Dr. M. Revel and Dr. O. Laub (InterPharm Laboratories, Rehovot, Israel) in a concentration of 1.13 mg/ml protein containing 43×10^6 IU/ml.
 - (4) Recombinant human IL-7 (rhIL-7) was
- 20

provided by Pepro Tech (New Jersey, USA) as lyophilized powder and was reconstituted to 100 mg/ml.

Lymphocytes were preactivated in vitro with rhIL2 or with combinations of rhIL2 and other cytokines for four days at 37°C. Concentrations of

5 cytokines used for in vitro incubation of lymphocytes were as follows: (a) rhIL2: 6000 IU/ml; (b) rIL6: 100-1000 U/ml; (c) rIL7: 10ng/ml; and (d) IFN γ : 1000 U/ml.

Anti-tumor effects of CAL collected post-culturing were assayed against natural killer (NK)-sensitive K562 tumor cells and NK-resistant Daudi tumor cells. The in vitro toxicity was evaluated by specific chromium release following incubation of effector cells with chromium-labeled tumor cells. Results are presented below as lytic units per 10⁶ effector cells, determined for 30% lysis of 5 x 10³ target cells:

Cytotoxicity (Lytic Units/10⁶ Cells)

	<u>Anti-Daudi</u>	<u>Anti-K562</u>
IL2 alone	48	53
IL2 + IL6 + IL7 + IFN γ	210	129